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13. ABSTRACT *(Maximum 200 Words)*

Marrow ablative doses of chemotherapy followed by stem cell rescue (MAT/SR) produce a high frequency of objective responses in patients with metastatic breast cancer, with up to 40-50% complete responses. Unfortunately, responses tend to be short-lived. Interleukin-2 (IL-2) can activate lymphocytes to kill multidrug resistant cancer cells. Our phase I data established that a single course of low-dose IL-2 (1.6 million IU/m2/day as a continuous i.v. infusion for 18 days) as consolidation treatment to patients with metastatic breast cancer early after MAT/SR. Seven patients (60%) remained in complete remission at a median of 435 days (range: 224 - 720 days) post stem cell transplantation. We are therefore performing a larger scale phase II trial of MAT/SR, followed by a single 18-day continuous infusion of low-dose IL-2 to activate lymphocytes to kill residual chemotherapy-resistant cancer cells. Disease free survival and toxicity assessment represent major clinical aims (Specific aim 1). Immunologic effector mechanisms induced following MAT/SR by IL-2 infusion will be evaluated using phenotypic and functional assays for LAK cell induction (Specific Aim 2). Accrual to this study has been delayed due to a change from a randomized trial to a single arm phase II study and due to negotiations between the University of Utah and the Army MRMC concerning liability clauses in the consent document. A finalized protocol has now been agreed upon.

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Introduction:

At least 46,000 women die from metastatic breast cancer each year in the United States. Median survival remains 12-18 months from the diagnosis of metastatic disease, and progression-free survival beyond 5 years is rare (<10%). This has led to the testing of escalated, marrow ablative doses of chemotherapy followed by stem cell rescue (MAT/SR). This approach produces a high frequency of objective responses in patients with metastatic breast cancer, with up to 40-50% complete responses. Unfortunately, responses tend to be short-lived, and only a minority of women (10-20%) achieve long-term disease free survival. Relapses may be due to both minimal tumor contamination of stem cells reinfused into patients, as well as residual chemotherapy resistant tumor cells not cleared by the MAT/SR regimen. IL-2 activated lymphocytes, termed lymphokine-activated killer (LAK) cells have significant cytotoxic activity against autologous breast cancer cells and breast cancer cell lines. Our own studies have demonstrated that multidrug-resistant tumor cells remain sensitive to LAK cell mediated killing. We have completed a phase I study to test the feasibility of administering a single course of low-dose IL-2 (1.6 million IU/m^2/day as a continuous i.v. infusion) as consolidation treatment to patients with metastatic breast cancer early after MAT/SR. This study established that IL-2 consolidation could be safely begun starting on day +14 post MAT/SR with minimal toxicity. Substantial LAK cell induction was observed, using flow cytometric and cytotoxicity assays. Thus far, only 3 of 10 patients have had breast cancer relapse or progression, and a small second breast cancer was detected in 1 patient. Seven patients (60%) remain in complete remission at a median of 435 days (range: 224 - 720 days) post stem cell transplantation. Because patients with metastatic breast cancer transplanted with active disease have a 60% and 80% probability of relapse at 1 year and 3 years, respectively (without IL-2), we are proposing to test this promising immunotherapy consolidation strategy in a single-institution randomized prospective trial. We propose to perform cytoreduction in patients with metastatic breast cancer using MAT/SR, followed by continuous infusions of low-dose IL-2 starting on day +14 to activate lymphocytes to kill residual chemotherapy-resistant cancer cells. Based on preliminary data, we hypothesize that a single course of IL-2 will result in a significant improvement in disease-free survival, with minimal toxicity. Effectiveness of this approach may correlate with the effective induction of LAK precursor and effector cells, as well as evidence for reduction in the burden of minimal residual cancer cells. In **Specific Aim 1** we propose to perform a prospective randomized clinical trial to test whether the addition of 1 cycle of continuous i.v. infusion of IL-2 in women with metastatic breast cancer, starting on day +14 after treatment with MAT/SR, can increase progression-free and overall survival by 25%. In **Specific Aim 2** we will evaluate possible immunologic effector mechanisms

induced following MAT/SR and IL-2 infusion. Phenotypic and functional assays for LAK cell induction and enzyme immunoassays for circulating pro-inflammatory cytokines will be performed. Following review by the US Army, **Specific Aim 3** (detection of residual tumor cells in bone marrow and stem cell products by flow cytometry and RT-PCR) was omitted

Body:

Accrual to this study has been delayed due to two unanticipated events. Shortly after this proposal was funded in 1999, a series of randomized trials was reported at the American Society of Clinical Oncology meetings comparing standard dose chemotherapy and marrow ablative therapy and stem cell rescue (MAT/SCR) for treatment of advanced breast cancer. The conclusions of all but one of these trials was that there was no advantage to stem cell transplants in breast cancer patients(1-3). The remaining trial (Bezwoda, et al) was found to contain fraudulent data(4). These findings made the proposed control arm of our randomized clinical trial (MAT/SCR alone) unacceptable. Since the goal of MAT/SCR in our trial was to provide maximal cytoreduction prior to IL-2 based immunotherapy, this goal was still felt scientifically reasonable, given our impressive phase I trial results. In order to further prove the validity of these observations, it was felt by Dr. Peterson and myself that a change from a randomized trial to a single arm phase II study (MAT/SCR followed by an 18 day infusion of IL-2) was warranted. This change was discussed with the USAMRMC and the study protocol and consent documents were rewritten. A second point holding up the clinical trials was due to negotiations between the University of Utah lawyers and the USAMRMC concerning required liability clauses in the consent document. After many months of negotiations, a finalized consent language and protocol have now been agreed upon. A final draft has been submitted to the University of Utah IRB. Once IRB approval and approval by the USAMRMC research safety officer have been obtained, the study will open to patient accrual (tentatively within 2 months). Having overcome these unanticipated delays, we remain enthusiastic to test the scientific hypothesis that IL-2 consolidation following MAT/SCR will produce long term disease free survival in 30-40% of metastatic breast cancer patients with acceptable toxicity.

Personnel required for the start of this clinical trial are all in place, ready to being patient accrual once IRB and USAMRMC approval are obtained. All methodologies required for patient IL-2 treatment and sample analysis for LAK cell induction have been worked out and are ready for use in this trial. Our proposal is to add an additional year onto study accrual, due to the delayed start of the trial, as a no-cost extension, using funds carried over from preceding years.

Task 1: Patient Enrollment: (months 1-36-delayed to month 14)

- Protocol will be presented to eligible patients prior to MAT/SCR.
- Patients will be randomized according to a random number generator computer program.
- Appropriate people/departments will be notified of patient enrollment and randomization, to include: site pharmacy, Dr. Wolf Samlowski and/or lab and Dr. Wayne Green and/or lab.
- A Progress Note will be entered into the patient's medical record regarding patient consent, enrollment, randomization, and the study requirements.

Task 2: Administration of IL-2: (months 1-36-delayed to month 14)

- On Day + 14, the patient will come to clinic (if discharged), where they will be have their vital signs taken and be seen by a physician extender for a baseline physical exam.
- The CADD-1 pump and supplies will be reviewed with the patient and caregiver(s).
- IL-2 will be started and the patient will remain in clinic for at least one hour to monitor vital signs and any adverse reactions.
- A patient diary will be given to the patient to help monitor and track fevers, other reactions, admissions, etc. (See attached sample.)
- Patient will be seen in clinic a minimum of once per week and also as needed. IL-2 cassettes will be changed every six days by the Research Nurse. Review of any adverse reactions or other problems will take place.

Task 3: Specimen Collection: (months 1-44-delayed to month 14)

- Approximately 50 cc's of blood will be collected in heparinized, green top tubes on Day 0, +7, + 14, +21, +32 and +100 and delivered to Dr. W. Samlowski's and Dr. W. Green's labs. (See below for details of lab procedures)
- Samples of pre-transplant and day 100 bone marrow material, as well as stem cell products will be transported to Dr. W. Samlowski's lab for evaluation of minimal residual tumor cells (5 ml marrow or PBSC cells in a heparinized syringe.

Task 4: Analysis of LAK cell induction (months 1-44-delayed to month 14)

- Samples will be analyzed for T cell and LAK cell markers by flow cytometry (Dr. Green's lab)
- Analysis of patient samples for LAK precursor and cytolytic cell function will be performed (Dr. Samlowski's lab)

Task 5: Assays for tumor cell detection in bone marrow and stem cell products (deleted

from funding by USAMRMC)

Task 6: Data Collection (months 1-48-delayed to month 14):

- The following will be collected while the patient is being followed by the Blood & Marrow Transplant team: priming dates, G-CSF dosing, BMT date, engraftment, side effects of IL-2 (fevers, rash, etc), infections, readmission, relapse, death and other significant events.
- At day 100, the patient will be seen by the physician to evaluate their disease and health status. Information such as infections, readmission, relapse and death will be collected.
- Patients will then be followed yearly or as needed to monitor disease status and/or death.

Task 7: Interim Analysis: (approximately month 36)

- After approximately 30 patients are enrolled, data collected from Dr. Wolf Samlowski's lab and Dr. Wayne Green's lab, together with information collected in the CRF's will be analyzed by the principal investigators.

Task 8: Final Analysis: (month 60)

- After enrollment of 60 patients is complete, data collected from Dr. Wolf Samlowski's lab and Dr. Wayne Green's lab and information collected in the CRF's will be analyzed by the principal investigators. At the completion of this study a report will be generated.

Key research accomplishments:

We treated 20 patients with MAT/SCR in our phase I pilot trial. Patients received IL-2 either starting on day 1 (10 patients) or day 14 (10 patients) following stem cell infusion. A total of 17 patients were evaluable for response at the time of initial analysis. A total of 17 patients (85%) completed the IL-2 course. Three patients receiving IL-2 from day 1 required IL-2 infusions to be terminated early (2 fever, 1 thrombocytopenia). Relapse free survival was 45% with 580 day median follow-up (135-1175 days), with 75% overall survival.

LAK cell activation was evaluated in patients undergoing IL-2 infusions starting either day 1 (5 patients) or day 14 post stem cell infusion (5 patients). Cytotoxicity against the MCF-7 breast cancer line was detected in all patients, regardless of whether the IL-2 infusion was started day 1 or 14. Increased cytolytic activity was detected in cytotoxicity assays performed with the addition of IL-2, suggesting a substantial increase in

circulating LAK cell precursors in both patient populations. Phenotypic evaluation established that while CD56+ cell populations were expanded in both patient groups, the absolute number of circulating CD56+ cells was 10-fold higher in patients receiving IL-2 starting on day 14.

Due to these results, our current clinical trial will treat patients beginning on day +14 with a 18 day infusion of IL-2 to verify these exciting clinical results in this high-risk breast cancer population.

Reportable outcomes:

We have published the results of our phase I trial in abstract form (copies enclosed):

1. Petersen FB and Samlowski WE. Low dose interleukin-2 (IL-2) therapy after marrow ablative therapy and peripheral blood stem cell rescue (MAT/SR) for metastatic breast cancer (M-BRCA): A phase I feasibility study. Blood 19:380b, 1999

2. Petersen FB and Samlowski WE. Low dose interleukin-2 (IL-2) therapy after marrow ablative therapy and peripheral blood stem cell rescue (MAT/SR) for metastatic breast cancer (M-BRCA) induces significant non-specific lymphokine activated killer (LAK) cell activity against the MCF-7 breast cancer cell line in vitro: Blood 19:57b, 1999

A manuscript for submission to a peer reviewed journal (J. Clinical Oncology) is currently being prepared.

Conclusions:

The proposed use of IL-2 following maximal cytoreduction of tumor by MAT/SCR remains promising based on our preliminary data, with 45% of patients achieving ~2year disease free survival. As soon as regulatory approval of the revised protocol and consent documents are obtained, we will confirm the effectiveness of this regimen in a larger scale phase II trial.

References:

1) Stadtmauer EA et al. Phase III randomized trial of high dose chemotherapy and stem cell support shows no difference in overall survival or severe toxicity compared to maintenance chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil

(CMF) for women with metastatic breast cancer who are responding to standard induction chemotherapy Proc ASCO 18:1a, 1999.

2) Peters W, et al. A prospective randomized comparison of two doses of combined alkylating agents as consolidation after CAF in high-risk primary breast cancer involving ten or more axillary lymph nodes: Preliminary results of CALGB 9082/SWOG9114/NCIC MA-13. Proc ASCO 18:1a, 1999.

3) Scandinavian Breast Cancer Study Group. Results of a randomized adjuvant breast cancer study with CTCb supported by autologous bone marrow stem cells versus dose-escalated and tailored FEC therapy. Proc ASCO 18:2a, 1999.

4) Bezwoda WR. Randomized controlled trial of high dose chemotherapy (HD-CNVp) versus standard dose (CAF) chemotherapy for high-risk, surgically treated primary breast cancer. Proc ASCO 18:2a, 1999.

Blood 19:3806, 1999

Abstract# 4925

LOW-DOSE INTERLEUKIN-2 (IL-2) THERAPY AFTER MARROW ABLATIVE THERAPY AND PERIPHERAL BLOOD STEM CELL RESCUE (MAT/SR) FOR METASTATIC BREAST CANCER (m-BRCA) - A PHASE I FEASIBILITY STUDY.

F. B. Petersen, W. E. Samlowski*. *University of Utah Health Sciences Center, Salt Lake City, UT.*

MAT/SR has by itself not resulted in any notable improvement when used in patients with active m-BRCA. However, MAT/SR will result in a maximum fraction of such pts. achieving a temporary period of clinical remission (CI-R) and potentially be important in setting the optimal stage for subsequent curative non-cross resistant therapies such as immunotherapy. IL-2 activated NK-cells exert a profound cytolytic activity against chemotherapy resistant BRCA cell lines in vitro. Thus, we hypothesize, that IL-2 given as consolidation after MAT/SR for m-BRCA will result in a more complete BRCA cell kill in vivo, and have a greater curative potential than MAT/SR without IL-2. We have concluded a clinical pilot trial of giving one or two 18 day cycles of continuos infusion low-dose IL-2 (1.8 x 10^6 U/m^2/24h) IV starting 14 days after a standard MAT/SR procedure in patients with active m-BRCA. The clinical results are listed in the table below. We determined that this IL-2 regimen resulted in m-BRCA patients developing lymphokine activated killer (LAK)- and LAK-precursor cells with cytolytic activity against chemotherapy resistant BRCA cell lines in vitro. We conclude that such a regimen is well tolerated with minimal to no side-effects, able to be given on an outpatient basis to a majority of pts., and that clinical outcome data from this trial suggests a possible benefit of IL-2. For this reason, we have initiated a phase III randomized trial in patients with active m-BRCA, who after a standard MAT/SR regimen (STAMP V), are randomized to receive or not to receive IL-2 in order to definitively test our hypothesis.

Outcome of giving IL-2 IV early after MAT/SR for m-BRCA (N=20)

	Completed IL-2 course	Have Relapsed	Are Alive	Alive in Clinical CR
Number of Patients (%)	17 (85%)	11 (55%)	15 (75%)	9 (45%)
Number of days from MAT/SR median (range)		180(0-790)	470(135-1175)	580(135-1175)

Abstract# 4926

OPPORTUNISTIC INFECTION (OI) IN RECIPIENTS OF CD34-SELECTED (CD34+) AND UNSELECTED (UNS) AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT) FOR NON-HODGKIN'S LYMPHOMA (NHL).

Jocelyne Y. Raad*,[1] David D. Hurd,[1] Julia Cruz,[1] James Perry,[2] James Radford,[3] William Dodge*,[1] Kevin P. High*,[1] *[1]Department of Internal Medicine, Section of Hematology/Oncology, Wake Forest University School of Medicine, Winston Salem, NC; [2]Yardley, PA; [3]Asheville, NC.*

CD34-selection has been associated with unusual OIs (cryptosporidiosis and *Pneumocystis carinii* pneumonia (PCP)) in recent reports of AHSCT recipients, perhaps due to a more profound cell-mediated immune deficit. To further evaluate this, we performed a retrospective cohort study of OI risk in patients with NHL who underwent dose-intensive therapy (DIT) followed by CD34+ AHSCT and compared them to a concurrent control patient group with NHL who received UNS AHSCT. Median follow-up was 300 days in the CD34+ group and 475 days in the UNS group. Baseline variables (age, gender, race) were well matched and there were no differences between groups in post-AHSCT chemotherapy. However, there was a trend toward earlier NHL recurrence (median disease free survival 248 days in the CD34+ group vs. 360 days in the UNS group), and significantly more ($p<0.001$) protocol-mandated use of cyclophosphamide/etoposide/ carboplatin/TBI as a preparative regimen in the CD34+ group.

Table 1. OIs in NHL Patients > 30 Days After CD34-selected or Unselected AHSCT

	CD34+ Group (n=27)	Unselected Group (n=34)
Serious OIs		
PCP	3	0
Invasive Aspergillosis	1	0
CMV Disease	1	0
Total	5 (19%)	0
Herpes Viral Illnesses		
Herpes simplex virus (HSV)	3	1
Zoster (HZ)	6	10
Total	9 (33%)	11 (32%)

The relative risk of PCP, 9.8 ($p=0.05$) or any serious OI, 16.9 ($p=0.02$) was higher in the CD34+ group. In addition, three CD34+ patients had multiple OIs (CMV and HZ; PCP and HZ; PCP, HZ and HSV) vs. none in the UNS group. The overall incidence of herpes viral infections did not differ.

Conclusion: OIs may occur more frequently in recipients of CD34-selected AHSCT. However, a larger study is needed to control for variables such as preparative regimen, length of follow-up and disease recurrence to definitively determine the role of CD34 selection in OI risk.

Abstract# 4927

SUCCESSFUL LUNG TRANSPLANTATION IN AN ADULT PATIENT WITH BRONCHIOLITIS OBLITERANS ASSOCIATED WITH GRAFT-VERSUS-HOST DISEASE AFTER MARROW TRANSPLANTATION.

W. Rabitsch*, E. Deviatko*, T. Birsan*, H. Loidolt*, A. Schulenburg*, F. Keil*, C. Herold*, G. Dekan*, K. Lechner, H. T. Greinix, W. Klepetko*, P. Kalhs.

Bone marrow transplantation-related bronchiolitis obliterans(BO) occurs in patients who develop extensive graft-versus-host disease (GVHD). If the disease does not respond to immunosuppressive therapy within 6 months the prognosis is dismal.

We present a 37-year old male with extensive GVHD following allogeneic bone marrow transplantation (BMT) from his HLA-identical sister because of chronic myelogenous leukemia in chronic phase. After conditioning therapy with busulfan and cyclophosphamide, 2.09x10^6 CD34+ cells/kg body weight were infused. GVHD prophylaxis consisted of cyclosporine A(CSA) and short course methotrexate. After hematological reconstitution the patient was discharged without signs of GVHD. Seven months after transplantation the patient presented clinically with persistend cough, inspiratory rales, bronchospasm and exertional dyspnea. Pulmonary function tests showed rapidly evolving severe airflow obstruction and hypoxemia without restrictive ventilatory defects(forced expiratory volume 1 second/vital capicity)(FEV1/VC:58%). Chest x-ray showed mild overinflation and was otherwise unremarkable. High-resolution computed tomography showed bronchial dilatation and a mosaic of attenuation. Bronchoalveolar lavage excluded bacterial, viral or fungal infection. Despite immunosuppressive and broad-spectrum antibiotic therapy his clinical status and his pulmonary function test worsened further (FEV1/VC:40%). Eight months after onset of GVHD a double-lung transplantation (LTX) was performed. Immunosuppressive therapy consisted of CSA, mycophenolate mofetil(MMF)and corticosteroids. The postoperative course of the patient was uneventful. At present, 29 months after BMT and 13 months after LTX, the patient is in complete hematological remission and has pulmonary function tests in the normal range(FEV1/VC:121%)enabling him to perform various sportive activities. Lung transplantation is now accepted as therapeutic option for a broad spectrum of otherwise incurable lung diseases. Selected patients with BO after allogeneic BMT who are refractory to conventional immunosuppressive therapy might widen this spectrum.

Abstract# 4928

SECOND ALLOGENEIC BONE MARROW TRANSPLANT AFTER RELAPSE OF HEMATOLOGIC MALIGNANCIES FOLLOWING FIRST AUTOLOGOUS OR ALLOGENEIC BONE MARROW TRANSPLANT.

T. Roberts*, Y. Koc*, K. Sprague*, H. Grodman, D. Mogavero*, E. Berkman, D. Schenkein, K. B. Miller. *Tuft's-New England Medical Center, Boston, MA.*

The role of allogeneic bone marrow transplant (allo-BMT) after relapse following an autologous or allogeneic bone marrow transplant is controversial. We analyzed the outcomes of patients at our center that have undergone a second allogeneic bone marrow transplant who relapsed after receiving an autologous or allo-BMT. Between September 1992 and July 1999, 22 patients underwent allogeneic bone marrow transplant (BMT2) for relapsed disease. Median follow-up after BMT2 was 4 months (range 0.4-82). Indication for 2nd allo-BMT included: AML (n=12), CML (n=2), Hodgkin's disease (HD) (n=3), non-Hodkgin's lymphoma (NHL) (n=3), multiple myeloma (MM) (n=1) and myelodysplastic syndrome (MDS) (n=1). First transplants (BMT1) included 9 allo-BMTs and 13 auto-BMTs. Second transplants (BMT2) included 8 matched unrelated donor (MUD) and 14 matched related donor. Preparative regimens for BMT2 included Bu/Cy (n=4), Cy/TBI (n=6), VP 16/Bu/Cy (n=8), VP 16/Cy/TBI (n=4). Patient characteristics: M:F=12:10; median age at time of BMT2=38 yrs (range 17-53 yrs); median time from BMT1 to BMT2=16 mo (range 6-84), from BMT1 to relapse= 10 mo (range 3-74), and from relapse to BMT2=4 mo (range 1-19). For all patients, the 2-yr disease-free survival (DFS) and overall survival (OS) was 42% and 24%, respectively. The 2-yr DFS for sibling-donor transplants was 45% and 2-yr OS=45%. For all patients with AML/MDS (n=13), the 2-yr DFS was 40% and OS 12%. For all other diseases (n=9), the 2-yr DFS was 50% and 2-yr OS = 33%. Median time to ANC>200 = 14 days (range 11-22) and median time to ANC>500 = 16 days (range 13-27). There were 5 peritransplant deaths. Five patients relapsed post-BMT2 with a median time to relapse of 5.5 months (range 3-12). Four of these 5 patients have died, with a median time from relapse to death = 37 days. Cause of death in patients in CR were multiorgan failure (1), pulmonary embolus/ARDS (1), *pseudomonas* pneumonia (1), venoocclusive disease (1) and chronic GVHD of the GI tract with sepsis (1). In univariate analysis for DFS, predictors of poor outcome included time from BMT1 to relapse <6 months ($p<0.001$) and time from BMT1 to BMT2 < 12 months ($p=0.002$). In conclusion, second allogeneic bone marrow transplant as treatment for relapsed hematologic malignancy is feasible and can achieve stable long term complete remissions in selected patients. Early relapse (less than 6 months from BMT1) and less than 12 months between first and second transplants predict for both poor DFS and OS. Patients who received an unrelated donor transplant had a poorer outcome than those patients with a matched-sibling related donor transplant.

Abstract# 4929

HIGH FREQUENCY OF ISOLATED EXTRAMEDULLARY RELAPSE IN PATIENTS TRANSPLANTED FOR ACUTE MYELOID LEUKEMIA.

G. De Rosa*, L. Pezzullo*, A. Lucania*, C. Selleri, B. Rotoli. *Department of Hematology, Federico II University Medical School, Naples, Italy.*

Extramedullary (EM) localizations of acute myeloid leukemia (AML) are infrequent at diagnosis and difficult to eradicate with conventional systemic chemotherapy (CHT). Aim of this study was to evaluate the frequency of EM relapse in patients <65 years treated with CHT alone in comparison with transplanted patients (either allogeneic or autoBMT). The two cohorts of patients were sufficiently similar for age and treatment protocol; no patient had EM sites involved at diagnosis. The CHT group was composed of 101 patients, most of them treated when BMT was not a routinary approach; median age 47y, range 12-65. In this group 47/101 patients relapsed; relapse was hematological in 43 (91%) and EM in 4 (9%). 60 patients were transplanted (median age 39y, range 10-65). 35 underwent alloBMT and 25 autoBMT; all were conditioned with BuCy2. In this group 22/60 patients relapsed (12/35 alloBMT and 10/25 autoBMT); relapse was hematological in 15 (68%) and extramedullary in 7(32%)($p=0.01$). Of the 7 EM relapses occurring in the BMT group, 4 were seen in the alloBMT group and 3 in the autoBMT group. The higher frequency of EM relapses in transplanted patients is probably due to insufficient eradication by the conditioning regimen. Possible interpretations include i) shorter treatment duration in transplanted versus non-transplanted patients; ii) easier abnormal homing of leukemic cells after myeloablative and immunoablative regimens; iii) less effective GvL effect in "sanctuary" sites. Sites of EM relapse were CNS and spine in alloBMT, CNS and skin (2 patients) in autoBMT; absence of skin relapse in alloBMT might support the relevance of GvH-associated GvL as protective mechanism, considering that skin is the most common site of GvH.

 Blood 19: 576, 1999

Abstract# 3406

CORD BLOOD LYMPHOCYTES HAVE A HIGH THRESHOLD FOR ACTIVATION BY PHORBOL ESTER. Isabel Perez-Cruz*,[1] Katarizna Bogunia-Kubik*,[1-2] Paul Fallen*,[1] Alejandro Madrigal,[3] Shara Cohen*.[1] *[1]Research, Anthony Nolan Research Institue, The Royal Free School of Medicine, London, United Kingdom; [2]Hirszfeld Institute of Immunology, Wroclaw, Poland; [3]Hematology, The Royal Free School of Medicine, London, United Kingdom.*

The use of cord blood (CB) instead of bone marrow (BM) has provided a promising alternative for stem cell transplantation. One advantage of CB may be that CB transplantation can cause less graft versus host disease (GvHD) than BM, although the mechanism for this is yet to be defined. In CB the T and natural killer (NK) cells have reduced function compared to adult cells and both these cell types are important effectors in GvHD. Since cytokine production is a major indicator of T cell and NK cell function and the cytokine cascade has been shown to be a major factor in GvHD perpetuation, we investigated the ability of T and NK cells within CB to make the pro-inflammatory cytokines IL-2, IFNγ and TNFα.

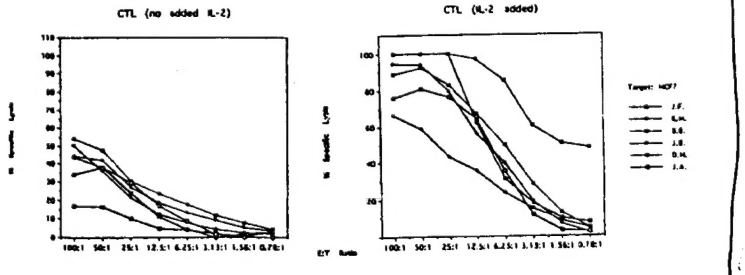
When mononuclear cell populations were stimulated with the mitogens ionomycin (I; 1μM) and phorbol-12-myristate-13-acetate (PMA; 20ng/ml) the frequency of IL-2, IFNγ and TNFα producing T (CD3+) and NK (CD3-) cells within the CB mononuclear cell population was reduced compared to the equivalent adult cell population (assessed by intracellular cytokine staining) (P< 0.001, 0.05, 0.02 for IFNγ , TNFα and IL-2, respectively in the T cells and 0.001, 0.001, 0.002 for IFNγ, TNFα and IL-2 respectively in the NK cells). It has previously been suggested that the reduced ability of CB mononuclear cells to make cytokine in response to PMA + I is due to a defect in CB cell signalling. However, we show that this is due to the naive cells (CD45RA+) in CB having a higher threshold of activation by PMA than adult mononuclear cells. Our results imply that there may be signalling differences upstream of Ca2+ and PKC activation, since naive T cells derived from CB could be stimulated by a high concentration of PMA (50ng/ml PMA + 1μM I enabled detection of >10% naive T cells making IL-2), but not by stimulation with immobilised anti-CD3. Our observation that CB derived lymphocytes require a higher threshold of activation by mitogen stimulation compared to adult cells, and that there may be signalling differences upstream of Ca2+ and PKC activation, may suggest a mechanism for the reduced GvHD after CB transplantation.

Abstract# 3407

LOW-DOSE INTERLEUKIN-2 (IL-2) GIVEN EARLY AFTER STEM CELL RESCUE (SR) FOLLOWING MARROW ABLATIVE THERAPY (MAT) FOR METASTATIC BREAST CANCER (M-BRCA) INDUCES SIGNIFICANT NON-SPECIFIC LYMPHOKINE ACTIVATED KILLER (LAK) CELL CYTOLYTIC ACTIVITY AGAINST THE MCF-7 BREAST CANCER CELL LINE IN VITRO. F. B. Petersen, W. E. Samlowski*. *University of Utah Health Sciences Center, Salt Lake City, UT.*

To investigate whether IL-2 given at low dose early after MAT/SR in patients (pts.) treated for m-BRCA resulted in any in vitro LAK cell activity, we gave 1.8 x 10^6 IU/m^2/24h as a continuos IV infusion to 10 pts. with advanced m-BRCA for 18 consecutive days. First marrow ablative doses of thiotepa, carboplatin and cyclophosphamide (STAMP V) were given, followed by IV infusion of previously harvested peripheral hematopoietic progenitor cells. IL-2 was started the day after the stem cell infusion, day + 1, (N=5) or 14 days after the stem cell infusion, day + 14 (N=5). Cell samples from 6 pts - 3 each from starting IL-2 day + 1 and + 14, were obtained 24 hours after finishing the IL-2 infusion and non-specific LAK cell cytolytic activity was induced in vitro against the MCF-7 breast cancer cell line in all patients (figure) and appeared similar regardless of whether IL-2 was started on day + 1 or day + 14. Increased cytolytic activity was noted when IL-2 was added indicating a substantial pool of circulating primed LAK precursor cells.

We conclude, that a low-dose regimen of continuos infusion IL-2 given over 18 consecutive days induces significant in vitro LAK cell cytolytic activity against cancer cell lines. As this potentially provides a non-cross resistant modality to overcome residual chemotherapy resistant breast cancer cells after MAT/SR in patients with advanced m-BRCA, we have proceeded to investigate whether this approach has any clinical efficacy.



Abstract# 3408

EXPRESSION PATTERNS OF HUMAN POLYCOMB-GROUP PROTEINS SUGGEST A REGULATORY ROLE FOR BMI-1/RING AND ENX/EED IN THE GERMINAL CENTER REACTION. Frank M. Raaphorst*,[1] Folkert J. van Kemenade*,[1] David P.E. Satijn*,[2] Arie P. Otte*,[2] Chris J.L.M. Meijer*.[1] (Intr. by Guy Sauvageau) *[1]Pathology, VU University Hospital, Amsterdam, The Netherlands; [2]EC Slater Institute, University of Amsterdam, Amsterdam, The Netherlands.*

Polycomb-group (Pc-G) proteins regulate embryonic development in Drosophila, mouse and man by inhibition of homeobox gene expression. Mouse Pc-G proteins are also essential for adult hematopoietic development, and contribute to cell-cycle regulation. Using immunohistochemical analyses, we show that human germinal center (GC) B-cell differentiation correlates with alternating and mutually exclusive expression of the human BMI-1 and ENX Pc-G proteins. The transition of resting mantle B-cells to rapidly dividing follicular centroblasts coincides with induction of Mib-1(Ki-67) expression, downregulation of BMI-1, and upregulation of ENX. Subsequent differentiation of centroblasts into centrocytes is reflected by loss of ENX and Mib-1 expression and re-appearance of BMI-1. Analysis of the RING and EED Pc-G proteins demonstrated that the RING expression pattern resembles that of BMI-1, while EED expression correlated with the ENX expression profile. These distinct expression patterns reflect the composition of two different human Pc-G complexes, one containing BMI-1 and RING, and the other ENX and EED. We speculate that antigen-specific development of B-cells in GC is guided by formation of different Pc-G complexes, with different functions and different target genes.

Abstract# 3409

ACTIVATION OF p120(ctn)/p55 IN EARLY B CELLS SUGGESTS A ROLE FOR THE CYTOSKELETON IN PRE-B CELL RECEPTOR-MEDIATED SIGNALING. Susan R. Rheingold*,[1] Junjie Fang*,[1] Stephan A. Grupp.[1] *[1]Pediatrics, Division of Oncology, The Children's Hospital of Philadelphia, Philadelphia, PA.*

Assembly of the precursor B cell receptor (pre-BCR) complex initiates downstream signaling essential for pre-B cell survival and development. Pre-BCR signaling is mediated by nonreceptor protein tyrosine kinases (PTKs) and their substrates. p120(ctn) is a catenin-related phosphoprotein which functions in transmitting signals initiated by cell-cell adhesion. It also is involved in binding to the actin cytoskeleton which in turn plays a role in intracellular signaling in mature B lymphocytes. The role of this system in early B cell signaling is unknown. Analysis of pervanadate/H_2O_2-stimulated progenitor (pro-) and pre-B cells showed that the presence of μ (IgM heavy chain) activated signaling via p120(ctn), identified in Western blots of cell lysates probed with anti-phosphotyrosine and anti-p120 antibodies. This activation was apparent both in pro-B and pre-B cells isolated from the bone marrow of mice expressing defined transgenic μ proteins as well as pro-B and pre-B cell lines transfected with μ proteins. p120(ctn) activation may highlight a role of this protein in adhesion signaling, presumably initiated by interaction of early B lymphocytes with the bone marrow stroma. Activation of tyrosine phosphorylation of p120(ctn) is further associated with activation of a 55kD tyrosine phosphoprotein. Western blot analysis of stimulated cell lysates shows that this 55kD protein is not blk, lck, fyn, or lyn, all PTKs known to be activated after ligation of the antigen receptor in mature B or T lymphocytes. This suggests that it may represent SH3P7, a 55kD cytoskeleton adapter protein. SH3P7 has been implicated in BCR-initiated signaling in mature B cells and further functions as an adapter protein to mediate association with the actin cytoskeleton. Activation of p120(ctn)/p55 in early B cells did not occur in the absence of μ protein, as seen in parent pre-B cell lines which do not express an endogenous μ protein. Association of the Igα/β heterodimer with the pre-BCR was not required for activation of p120(ctn)/p55 as shown in experiments utilizing mutant μ proteins which produce an impaired pre-BCR complex without Igα/β. These results point to a role of the actin cytoskeleton in pre-BCR mediated signaling, a role which is independent of the requirement for the Igα/β heterodimer.

Abstract# 3410

CONSTITUTIVE AND SELECTIVE EXPRESSION OF CYCLOOXYGENASE-2 IN MEDULLARY EPITHELIAL CELLS OF HUMAN THYMUS. B. Rocca*, N. Maggiano*, A. Habib*, L. Lauriola*, R. Landolfi*, F. O. Ranelletti*. (Intr. by Joel S. Bennett) *Dept. of Internal Medicine, Histology and Pathology, Catholic University School of Medicine, Rome, Italy; INSERM Laboratories, Paris, France.*

Cyclooxygenase (COX) -2 isozyme, which catalyzes the conversion of arachidonic acid to prostaglandins (PG), has been shown to be selectively expressed in a subset of medullary epithelial cells of the mouse thymus and to regulate $CD4^+$ lymphocyte maturation through PGE_2 (Rocca et al, *J Clin Invest* 1999). Furthermore, in mouse thymic medullary epithelial cell lines, COX-2 expression is modulated by interferon-γ and regulates the adhesion of $CD4^+$ lymphocytes (Rocca et al, *J Immunol* 1999). We investigated whether the two isozymes COX-1 and -2 are present in the human thymus and their pattern of expression. Thymus biopsies were obtained from 1- to 30-mo. old infants during open-heart surgery. Thymus sections were stained with polyclonal antibodies specific for COX-1 or -2. A strong positivity for COX-2 was selectively localized in the thymic medulla. Cells expressing COX-2 showed large nuclei with an ovoid shape and large cell bodies with cytoplasmic prolongations. These cells were sparsely localized in the medulla and showed no preferential proximity to Hassal's bodies. To further identify the phenotype of these cells, thymus sections were double stained for COX-2 and different antigens. COX-2 positive cells did not stain for the macrophagic marker CD68 or for the S-100 protein which identifies dendritic cells. On the other hand, COX-2 expressing cells were positive for cytokeratin and for HLA-DR molecules. These findings indicate that COX-2 is expressed in a subset of epithelial cells. We also stained sections of thymuses obtained from infants affected by Down syndrome (age range 1- to 30-mo. old) during open-heart surgery, which are characterized by a deficient T-cell maturation. No stain for COX-2 was observed in thymuses from Down children. COX-1 showed a completely different pattern of expression in normal thymuses. COX-1 positivity was almost exclusively localized in the periphery of the cortex. Cells positives for COX-1 were morphologically similar to thymocytes, showing a relatively smaller size, round shape and round nucleus. In conclusion, COX-2 appears to be constitutively and selectively expressed in a subset of medullary epithelial cells of the human thymus. These cells are positive for HLA-DR molecules, suggesting that they may be involved in the selection processes of mature T-lymphocytes. Thymuses from patients with Down syndrome, in which the maturation of T-cells is impaired, do not express COX-2.